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The effect of *in vitro* gamma radiation on *Anisakis* sp. larvae collected from the pink cusk-eel, *Genypterus brasiliensis* Regan, 1903

O efeito *in vitro* da radiação gama em larvas de *Anisakis* sp. coletadas do congro rosa, *Genypterus brasiliensis* Regan, 1903

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Abstract

The aim of this research was to study the impact of *in vitro* gamma radiation on *Anisakis* sp. larvae collected from *Genypterus brasiliensis* assessing the use of this technological process in the prevention of human anisakiasis. The parasites were irradiated with doses of 1, 3 and 6 kGy in a 0.65% NaCl solution and kept chilled between 5ºC and 8°C. The most effective dose used was 6 kGy, in which these parasite survived for a maximum period of 14 days.

Keywords: irradiation, *Anisakis* sp., *Genypterus brasiliensis.*

Resumo

A objetivo desta pesquisa foi estudar o impacto da radiação gama *in vitro* sobre as larvas de anisaquídeos coletadas do congro-rosa, *Genypterus brasiliensis* verificando a utilidade deste processamento tecnológico como controle para evitar a anisaquiose humana. Os parasitos foram irradiados com dosagens de 1, 3 e 6 kGy, mantidos em solução fisiológica 0,65% de NaCl e armazenados sob refrigeração a temperaturas entre 5ºC a 8°C. A mais efetiva dosagem usada foi a de 6 kGy, na qual as larvas desses nematóides sobreviveram por um período máximo de 14 dias.

Palavras-chave: irradiação*, Anisakis, Genypterus brasiliensis.*

Introduction

Nematodes from the Anisakidae family, especially those of the genus *Anisakis*, *Pseudoterranova* and *Contracaecum* are the fish helminths with the greatest zoonotic significance (Adams et al., 1997). The ingestion of raw or improperly processed fish infected with larvae in the third or fourth stage of these parasites can cause anisakiasis in humans (Acha and Szyfres, 1986; Sakanari and McKerrow, 1989; Adams et al., 1997). Ubeira et al. (2000) state that in 97% of the diagnosed cases of this illness were due to larvae of *A. simplex*.

Van Thiel et al. (1960) describe the first case of human anisakiasis in the Netherlands. Various cases of this illness have been recorded in Europe, America and, most especially, Asia, (Kliks, 1983; Matsuoka et al., 1994; Bouree et al., 1995; Muraoka et al., 1996; Rosales, 1999).

In anisakiasis, the parasites penetrate the gastro-intestinal tract, causing ulcerations and an inflammatory process that results in eosinophilic granuloma (Kliks, 1983; Muraoka et al., 1996; López Sabater and López Sabater, 2000).

In recent years, several authors have reported that allergic reactions can be unleashed in susceptible individuals after the ingestion of fish infected with anisakis larvae, even if these larvae have been killed by some type of technological processing, such as boiling or freezing (Audicana et al., 1997; Alonso et al., 1999; Purello D'Ambrosio et al., 2000; Garcia-Bara et al., 2001; Audicana et al., 2002).

Socio-cultural factors have a significant influence on the transmission of anisakiasis in certain populations. Types of cooking such as sushi and sashimi, ceviche, "green-herring", "lomi-lomi" and "Koi pla" that are based on raw or undercooked fish are potential foci of parasitic infections in humans, (Sakanari and Mckerrow, 1989; Adams et al., 1990). Oshima (1987) reports that in Japan alone, where this practice is very common, more than 2000 cases per year of this illness are registered.

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In Brazil, there are currently no records on anisakiasis, though research has detected the presence of larvae from the *Anisakidae* family in commercially important teleostean fish captured in marine waters (Barros and Amato, 1993; São Clemente et al. 1995; Barros and Cavalcanti, 1998; Alves and Luque, 2001; Silva and São Clemente, 2001; Alves et al. 2002).

Due to the threat that this zoonosis represents to public health, various studies have been carried out using various technological processes, such as boiling, refrigeration, freezing, smoking and salting, all of which have the purpose of rendering the *Anisakis* sp. larvae capable of infecting humans inactive (Gustafson, 1953; Deardoff and Throm, 1988; São Clemente et al., 1994; Karl et al., 1995; Marques et al., 1995; São Clemente et al., 1996).

Loaharanu and Murrel (1994) state that in many countries where it is usual to consume plates of raw or semi-processed animal based foods, the use of ionising radiation has been shown to be an alternative method of controlling helminth or protozoan parasites.

Van Mameren and Houwing (1968) and Oishi et al. (1972) studied the effects of gamma radiation in association with salting to make *Anisakis* sp. larvae inactive. Chai et al. (1993) researched the viability of the infection of these nematodes by exposure to gamma radiation using rats and rabbits as model animals.

The pink cusk-eel, *Genypterus brasiliensis*, is a fish of important commercial value. It is both consumed internally in Brazil and exported to other countries. Alves et al. (2002) record the presence of *Anisakis* sp. in the mesentery and visceral organs of this species.

The possibility exists of studying new technologies that prevent the pathogenic actions of these parasites, thereby providing consumers with increased food safety. The aim of this study was to measure the survival time of *Anisakis* sp. larvae collected from *G. brasiliensis* irradiated *in vitro*, verifying the efficiency of this technological process as a method of controlling anisakiasis.

Material and methods

Between October 2002 and September 2003, thirty-eight specimens of *Genypterus brasiliensis*, 41.5 – 93 cm of total length, were acquired in fish markets and shops in the counties of Niterói and Rio de Janeiro. They were transported in thermal boxes, packed with ice, to the Laboratório de Helmintos Parasitos de Vertebrados do Instituto Oswaldo Cruz, Departamento de Helmintologia, Fundação Oswaldo Cruz. The fish were identified according to Figueiredo and Menezes (1978). Filets were removed through an incision from close to the gill-covers to the caudal fin. The internal organs were transferred to Petri dishes containing a 0.65% NaCl solution and observed with the stereomicroscope to detect possible parasites. The filets were observed with a negatoscope to detect the presence of *Anisakis* sp. larvae following Myers (1979). The helminths were collected from the mesentery, the surface of the internal organs and the musculature. Nematodes were transferred to Petri dishes containing a 0.65% NaCl solution and kept chilled between 5º and 8°C. The parasites that were to be exposed to gamma radiation were transported in thermal containers, kept between 5º and 8°C, to the Laboratório de Instrumentação Nuclear da Coordenação de Programas de Pós-Graduação em Engenharia da Universidade Federal do Rio de Janeiro. A total of 70 *Anisakis* sp. larvae were divided into four groups: the control group of 25 parasites, divided into sets of 3 – 6 specimens and stored under temperatures between 5º and 8°C. The other three groups were exposed to gamma radiation in the Gammacell 220 Nordion irradiator, using ^{60}Co – 80Gy/min, with 7000 Ci activity, and dosages of 1, 3 and 6 kGy, respectively. These groups were made up of 15 parasites and divided into sets of 3 – 6 specimens to be irradiated. The nematodes were observed through a stereomicroscope on a daily basis to determine the mortality rate. These parasites were considered dead when they no longer showed any movement and they changed from a transparent colour to an opaque white. The nematodes that remained alive were kept in Petri dishes containing a 0.65% NaCl solution and stored between 5º and 8°C. After the death of the nematodes, they were fixed in AFA, dehydrated by alcoholic sequence, clarified with Aman's lactophenol and creosote and mounted in Canadian balsam, following Amato et al. (1991). The taxonomic identification of the Anisakidae larvae was based on the works of Hartwich (1974) and Rego et al. (1983) using a bright field microscope.

Results

In the control group the shortest period of survival of *Anisakis* sp. larvae was of 13 days, while the longest period was of 95 days (Table 1).

Table 1 – Survival time (days) of 25 *Anisakis* sp. larvae used in the control group, collected from *Genypterus brasiliensis* between October 2002 and September 2003

Control Group			
***Anisakis* sp. larvae no.**	Survival time (days)	***Anisakis* sp. larvae no.**	Survival time (days)
1	37	**14**	40
2	38	**15**	74
3	41	**16**	95**
4	42	**17**	45
5	24	**18**	47
6	28	**19**	50
7	38	**20**	54
8	59	**21**	73
9	30	**22**	83
10	31	**23**	25
11	87	**24**	40
12	30	**25**	47
13	13*		

* *Anisakis* sp. larva with shortest survival time.
** *Anisakis* sp. larva with longest survival time

Anisakis sp. larvae submitted to gamma radiation with dosages of 1, 3 and 6 kGy survived for different periods depending on the doses used, though only one specimen irradiated with a 6 kGy dose died after exposure to gamma radiation (Table 2).

The experiment showed that the survival time of *Anisakis* sp. larvae declined considerably as the dosage of gamma radiation was increased. The average survival time of these nematodes for the control group and those submitted to dosages of 1, 3 and 6 kGy were of 46.8, 30.9, 14.8 and 5.0 days respectively (Table 3).

Table 2 – Survival time (days) of 45 *Anisakis* sp. larvae, divided into three groups of 15 specimens exposed to gamma radiation with doses of 1, 3, and 6 kGy collected from *Genypterus brasiliensis* between October 2002 and September 2003

GAMMA RADIATION DOSAGES					
1 kGy		**3 kGy**		**6kGy**	
***Anisakis* sp. larvae no.**	Survival time (days)	***Anisakis* sp. larvae no.**	Survival time (days)	***Anisakis* sp. larvae no.**	Survival time (days)
1	18	**1**	4	**1**	0*
2	40	**2**	18	**2**	1
3	49**	**3**	18	**3**	3
4	16*	**4**	19	**4**	14 **
5	18	**5**	19	**5**	5
6	29	**6**	20	**6**	5
7	41	**7**	3*	**7**	7
8	43	**8**	6	**8**	13
9	30	**9**	4	**9**	3
10	43	**10**	6	**10**	5
11	20	**11**	10	**11**	6
12	23	**12**	13	**12**	6
13	27	**13**	43**	**13**	2
14	28	**14**	19	**14**	2
15	39	**15**	20	**15**	3

* *Anisakis* sp. larva with shortest survival time.
** *Anisakis* sp. larva with longest survival time

Table 3 – Statistical analysis of survival time of 70 *Anisakis* sp. larvae collected from *Genypterus brasiliensis* between October 2002 and September 2003 submitted to the different dosages of gamma radiation (kGy)

Dosages (kGy)	N	Survival Time (days)				
		Average	**Standard Deviation**	**Minimum**	**Maximum**	**Range**
0	25	46.8	21.01	13	95	82
1,0	15	30.9	10.81	16	49	33
3,0	15	14.8	10.27	3	43	40
6,0	15	5.0	3.98	0	14	14

N = Number of *Anisakis* sp. larvae analysed.

Discussion

The results of this experiment in relation to the temperature can be compared with the study of Marques et al. (1995), that showed that infected anisakids larvae survived for up to 34 days in specimens of the swordfish, *T. lepturus*, kept at cool temperatures (around 0°C).

Van Mameren and Houwing (1968) and Oishi et al. (1972) state that doses of up to 6 kGy are not capable of killing all *Anisakis* sp. larvae, either *in vitro* or present in the viscera and musculature of teleostean fish, agreeing with our results.

Van Mameren and Houwing (1968) studied the affect of gamma radiation in association with salting to make *Anisakis* sp. larvae inactive, and also irradiated these parasites *in vitro* in different concentrations of NaCl solution. The mortality rates obtained for the control groups and those submitted to 3 and 6 kGy doses in saline concentrations of 0%, 3%, 6% and 8%, were 72%, 4%, 25% and 86% respectively for the non-irradiated group; 20%, 5%, 55% and 70% for the group exposed to the 3 kGy dose; 50%, 55%, 45% and 55% for the group irradiated with the 6 kGy dose. These results differ from those obtained in our study, only scheduled to verify the impact of gamma radiation on these parasites, when only one out of the fifteen larvae irradiated *in vitro* and kept in a 0.65% NaCl solution died after a 6 kGy dose of irradiation .

Oishi et al. (1972) showed that *Anisakis* sp. larvae irradiated *in vitro* with a 6 kGy dosage, using solutions with 3% and 6% concentrations of NaCl, survived for a maximum time of five days, differing from this study, when the nematodes irradiated with the same dose and conserved in a 0.65% NaCl solution survived for up to 14 days.

Conclusion

The doses of gamma radiation used do not guarantee food safety for the consumer, since *Anisakis* sp. larvae remained alive after irradiation.

Therefore, it is obvious that public health authorities must adopt specific rules and procedures to detect the presence of, and control, these helminths, due the several cases of anisakids in teleostean fish purchased in Brazil (Barros and Amato, 1993; São Clemente et al. 1995; Barros and Cavalcanti, 1998; Alves and Luque, 2001; Silva and São Clemente, 2001; Alves et al. 2002).

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